13:24

REMARKS

Applicant notes that the election of the Claims in Group I (Claims 1, 4-9, and 13-17) made in the Response dated April 20, 2001, has been recorded. Thus, Claims 1, 4-9, and 13-17 were pending in the present application. Applicant added dependent Claims 18-21 in the Response faxed May 15, 2002. The Examiner has withdrawn Claims 2, 3 and 10-12, as being directed to a non-elected Group. In the present Response, Claims 16-19 have been cancelled without prejudice and new Claims 22 and 23 have been added. These new Claims are directed toward the CP1 nucleic acid and amino acid sequences. As these Claims find more than sufficient support in the Specification, these Claims do not contain new matter. Thus, Claims 1, 4-9, and 13-15, 20-23 are currently pending.

Applicant note that the Examiner has indicated that some references included in the PTO-1449 form have not been entered into this case. As these references were included solely to show the state of the art in general, Applicant is not submitting these references for the Examiner to enter into the record. As the Examiner is likely wellaware, these references provide techniques and other general information known to those in the art.

While the Examiner has removed multiple objections, one objection to the recitation of "wpr protease" in Claims 15, 17 and 21, has been maintained. The "wpr" abbreviation refers to "cell wall associated" protease. Applicant submits that the wpr protease referred to in the present Specification is the same as the "wprA" enzyme described in Margot et al. (Margot et al., Microbiol. 142:3437-3444). Thus, Applicant submits that the terminology in the Claims is definite.

The Examiner has also objected to Claim 13 as being grammatically incorrect. Applicant appreciates the Examiner's suggestion and have amended the Claim to correct the grammar in the Claim. The Examiners rejections of the Claims are addressed in the order below:

Claims 20 and 21 stand rejected under 35 U.S.C. §112, second 1) paragraph, as allegedly being indefinite;

13:24

- Claims 16-19 stand rejected under 35 U.S.C. §112, first paragraph, as 2) allegedly not meeting the written description requirement;
- Claims 1, 4-9, and 13-21 remain rejected under 35 U.S.C. §112, first paragraph as allegedly not meeting the enablement requirement; and
- Claims 1, 4-9, and 13-21 remain rejected under 35 U.S.C. §102(b) as 4) allegedly being anticipated by WO89/10976;

The Claims Are Definite 1)

The Examiner has rejected Claims 20 and 21 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. Applicant has amended the Claims without prejudice in order to more clearly indicate that SEQ ID NO:1 corresponds to the gene encoding cysteine protease 1 (SEQ ID NO:2). Applicant believes that the Claims are in condition for allowance and respectfully request that this rejection be withdrawn.

The Present Specification and Claims Meet the Written 2) **Description Requirement**

The Examiner has maintained his rejection of Claims 16-19 under 35 U.S.C. §112, first paragraph, as allegedly not meeting the written description requirement. While Applicant must respectfully disagree, in order to further the prosecution of the present application and Applicant's business interests, yet without acquiescing to the Examiner's arguments, Applicant has cancelled Claims 16-19. Applicant reserves the right to pursue these Claims in another application. As these Claims have been cancelled, this rejection is most and Applicant respectfully requests that this rejection be withdrawn.

The Present Specification and Claims Meet the 3) Enablement Requirement

The Examiner has maintained his rejection of Claims 1, 4-9, and 13-21 under 35 U.S.C. §112, first paragraph, as allegedly not meeting the written description requirement. More particularly, the Examiner argues that the Specification does not

09/30/2002

U.S. Serial No. 09/462,846

support the broad scope of the Claims. The Examiner argues that the Specification does not establish:

- regarding claims 16 and 17, the sequences of CP1 polypeptides or A) encoding polypeptides of all gram positive microorganisms, guidance for isolating said sequences from all gram-positive microorganisms, or the predictability that a CP1 gene is present in all gram-positive microorganisms;
- regions of a CP1 from any gram-positive microorganism, the polypeptide of SEQ ID NO:2, or the polynucleotide of SEQ ID NO:1 that may be mutated with an expectation of obtaining the desired biological activity;
- regions apr, npr, epr, wpr and mpr from all gram-positive microorganisms C) or Bacillus hosts that may be mutated with an expectation of obtaining the desired biological activity;
- the predictability that all gram-positive microorganisms or Bacillus hosts D) will possess a gene encoding SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1 as an undue amount of experimentation would be required to examine all gram-positive microorganisms for the presence of a gene encoding SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1; and
- the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Applicant must respectfully disagree with the Examiner's arguments. With regard to argument A), Applicant submits that the CP1 polypeptide sequences and nucleic acid sequences encoding CP1 polypeptide are indeed disclosed in the Specification as SEQ ID NO:2 and SEQ ID NO:1. Applicant further submits that there is no requirement that Applicant provide predictability as to whether all gram-positive microorganisms contain CP1. The Claims are only directed towards those microorganisms that DO contain CP1. Nonetheless, as indicated above, Claims 16 and 17 have been deleted without prejudice. Therefore, this rejection is moot as to these Claims.

In regard to arguments B and C), Applicant submits that any mutation or deletion that results in the inactivation of CP1 proteolytic activity alone, or in combination with mutations or deletions in apr, npr, epr, wpr, and/or mpr is intended. Applicant is not required to provide each and every mutation or deletion that would result in inactivation.

13:24

The Specification as filed provides means to identify CP1, as well as the nucleic acid and amino acid sequences of CP1, and means to assess proteolytic activity (See, pages 5-9 and 12). The additional proteins are known in the art (See e.g.,page 9).

In regard to D), Applicant submits that there is no requirement that Applicant show the predictability that all gram-positive microorganisms or Bacillus hosts will possess a gene encoding SEQ ID NO:2 or the polynucleotide of SEQ ID NO:1. Applicant respectfully submits that no undue amount of experimentation would be required to examine gram-positive microorganisms of interest for the nucleic acid sequence of SEQ ID NO:1, or the amino acid sequence of SEQ ID NO:2. Indeed, the present Specification provides means to determine the homology between the CP-1 of SEQ ID NO:1 and other sequences (See e.g., page 12). Furthermore, the amino acid sequence of SEQ ID NO:2 would be relatively easy to compare with other proteases.

In regard to E), Applicant is unsure as to what the Examiner is referring to in the dause "which of the essentially infinite possible choices is likely to be successful." Thus, Applicant cannot address this argument.

Nonetheless, in order to further the prosecution of the present application and Applicant's business interests, yet without acquiescing to the Examiner's arguments, Applicant has amended Claims 1, 13, and 14, and cancelled Claims 4 and 5. With regard to Claim 13, Applicant submits that the Bacillus host cell may be any species of Bacillus, as this Claim is directed toward transformed cells. Support for these amendments is provided throughout the Specification and no new matter has been added. Applicant reserves the right to pursue the originally filed, similar and/or broader Claims in another application(s). Applicant respectfully submits that the pending Claims are in condition for allowance and request that this rejection be withdrawn.

The Claims are Novel 3)

The Examiner has maintained his rejection of Claims 1, 4-9, and 13-17, under 35 U.S.C. §102(b) as being allegedly anticipated by WO 89/10976. The Examiner argues that "applicants have provided no evidence to distinguish CP1 or the polypeptide of SEQ ID NO:2 encoded by SEQ ID NO:2 from the cysteine protease of WO89/10976 nor have applicants distinguished the cysteine protease-deficient AP*/NP*B. subtilis mutant of WO89/10976 from the claimed microorganisms . . . While applicants have amended independent claims 1, 13, 18, and 20 to recite the limitation of SEQ ID NO:2 or SEQ ID

NO:1, this limitation does not distinguish the claimed microorganisms and methods of use thereof from the cited prior art as the cysteine protease-deficient AP*/NP* B. subtilis of the prior art would inherently have a mutated sequence of SFQ ID NO:1 due to homologous recombination resulting in inactivation of cysteine protease activity." (Office Action, pages 7-8). Applicant must respectfully disagree.

WO 89/10976 teaches a B. subtilis strain that is deficient in both alkaline protease and neutral protease, as well as a sulfhydryl-dependent residual cysteine protease and/or a residual serine protease activities. These residual proteases are described as providing residual protease activity in Bacillus strains that are apr/npr, and are responsible for the degradation of proteins in cultures of B. subtilis.

As previously indicated, there is no teaching in WO 89/10976 of an organism with a mutation or deletion of part or all of the gene encoding CP-1. Indeed, there is no sequence information provided in this publication for any cysteine protease. Applicant submits that the Examiner has provided no evidence that what is described in WO89/10976 is the same as the sequence set forth in SEQ ID NOS: 1 or 2. Indeed, since there are no sequences of any cysteine protease disclosed in WO89/10976, there is no evidence that can support the Examiner's argument. A search of Genbank for each of the inventors named on WO89/10976 failed to identify any sequence information submitted by any of the named inventors regarding a cysteine protease from B. subtilis. Furthermore, a search of the Merops database provided numerous cysteine proteases from B. subtilis, any of which could conceivably be the same as the WO89/10976 putative cysteine protease. A copy of this printout is attached hereto.

Applicant respectfully submits that:

"[t]he fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. In re Rijckaert, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993)." (MPEP 2122, emphasis original).

Furthermore, Applicant respectfully submits:

"'[t]o establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not

13:24

09/30/2002

sufficient." In re Robertson, 169 F.3d 743, 745, 49 USPQ2nd 1949, 1950-151 (Fed. Cir. 1999)." (MPEP 2112).

Thus, Applicant respectfully submits that there is simply no teaching nor suggestion in WO 89/10976 of the CP1 of the presently claimed invention. Likewise, there is no teaching in WO 89/10976 of an organism with such a mutation or deletion in CP-1, as well as mutation(s) and/or deletion(s) in at least one of the genes encoding apr, npr, epr, wpr, and/or mpr. Thus, WO 89/10976 does not teach each and every element of the Claims¹, a requirement for a reference to be anticipatory. Nonetheless, in order to further the prosecution of the present application and Applicant's business interests, yet without acquiescing to the Examiner's arguments, the independent Claims have been amended to recite SEQ ID NO:2. Applicant reserves the right to pursue the originally filed and/or broader Claims in other application(s). Applicant respectfully requests that this rejection be withdrawn and the Claims passed to allowance.

CONCLUSION

All grounds of rejection and objection of the Office Action of January 22, 2002, having been addressed, reconsideration of the application is respectfully requested. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicant encourages the Examiner to call the undersigned.

Respectfully submitted,

Dated: September 30, 2002

Registration No. 38,230

Genencor International, Inc. 925 Page Mill Road Palo Alto, CA 94304-1013 Phone: (650) 846-5838

Facsimile: (650) 845-6504

[&]quot;Anticipation is established only when a single prior art reference discloses, expressly or under principles of inherency, each and every element of a claimed invention." RCA Corp. v. Applied Digital Data Sys., Inc., 730 F.2d 1440, 221 USPQ 385, 388 (Fed. Cir. 1984).

GENENCOR LEGAL → 17038729307

U.S. Serial No. 09/462,846

APPENDIX I

MARKED-UP VERSION OF SPECIFICATION'S REPLACEMENT PARAGRAPHS AND REWRITTEN, ADDED, AND/OR CANCELLED CLAIMS

The following is a marked-up version of the Specification's replacement paragraphs pursuant to 37 C.F.R. §1.121(b), as well as a marked-up version of the Claims pursuant to 37 C.F.R. §1.121 (c)(1)(ii) with instructions and markings showing changes made herein to the previous version of record of the specification and claims. Underlining denotes added text while bracketing denotes deleted text.

IN THE CLAIMS:

Please cancel Claims 4, 5, and 16-19.

Please amend the Claims as follows:

- 1. (Twice Amended) A [gram-positive microorganism] <u>Bacillus subtilis</u> having a mutation or deletion of part or all of the gene encoding cysteine protease-1 CP1, wherein said gene encodes the amino acid sequence set forth in SEQ ID NO:2, and said mutation or deletion results in the inactivation of the CP1 proteolytic activity.
- 13. (Thrice Amended) A method for the production of a heterologous protein in a <u>transformed Bacillus</u> host cell comprising the steps of:
 - (c) obtaining a *Bacillus* host cell comprising <u>a</u> nucleic acid encoding said heterologous protein wherein said host cell contains a mutation or deletion in at least one of the genes encoding *B. subtilis* cysteine protease 1, wherein said at least one of the genes encoding cysteine protease 1 encodes the amino acid sequence set forth in SEQ ID NO:2; and
 - (d) growing said Bacillus host cell under conditions suitable for the expression of said heterologous protein.
- 14. (Twice Amended) The method of Claim 13 wherein said Bacillus host cell is selected from the group consisting of Bacillus subtilis, B. licheniformis, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquefaciens, B. coagulans, B. circulans, B. lautus, and B. thuringiensis.

13:24

20. (Amended) The method of Claim 13, wherein said [Bacillus, comprises the nucleic acid sequence set forth in SEQ ID NO:1] at least one of the genes encoding cysteine protease 1 comprises the nucleic acid sequence set forth in SEQ ID NO:1.

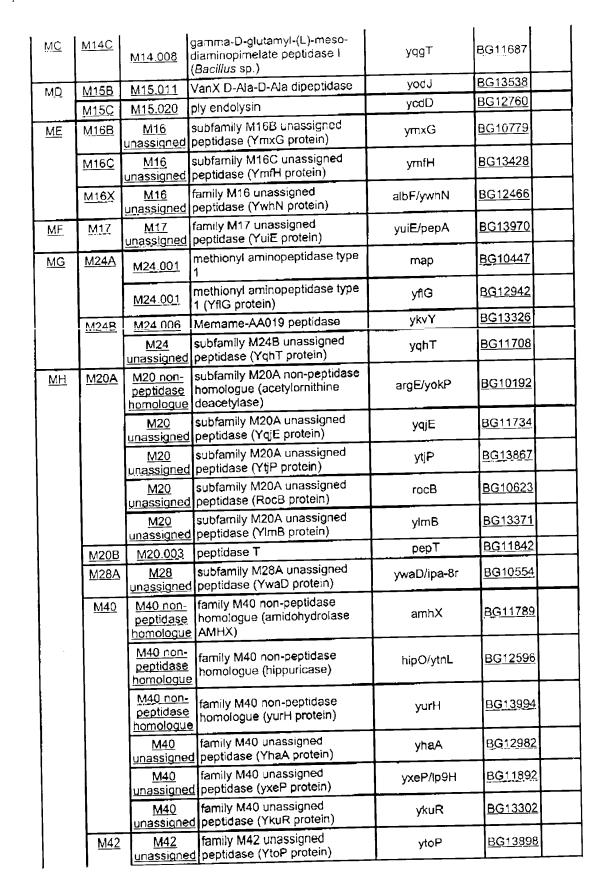
Please add the following new Claims:

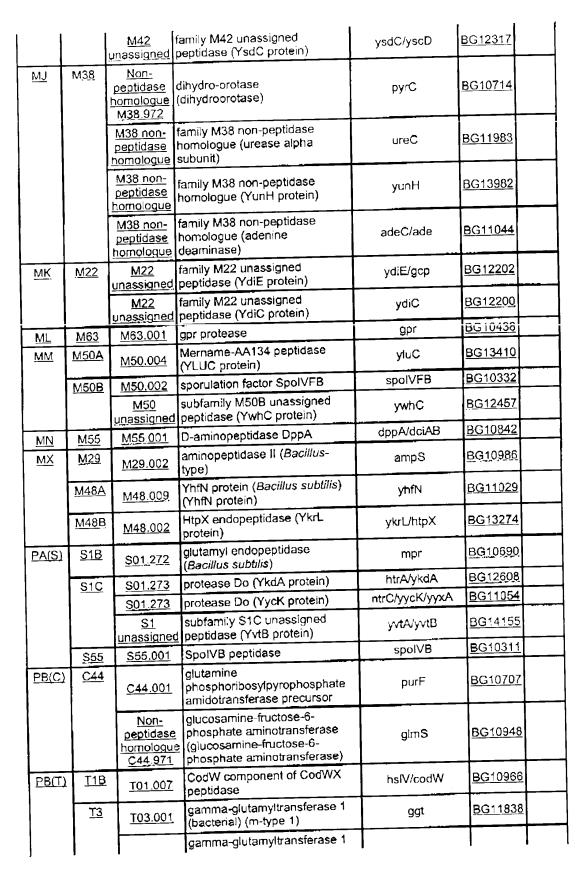
- 22. A Bacillus subtilis cysteine protease-1 encoded by a nucleic acid sequence comprising SEQ ID NO:1.
- 23. A *Bacillus subtilis* cysteine protease-1 set forth in an amino acid sequence comprising SEQ ID NO:2.

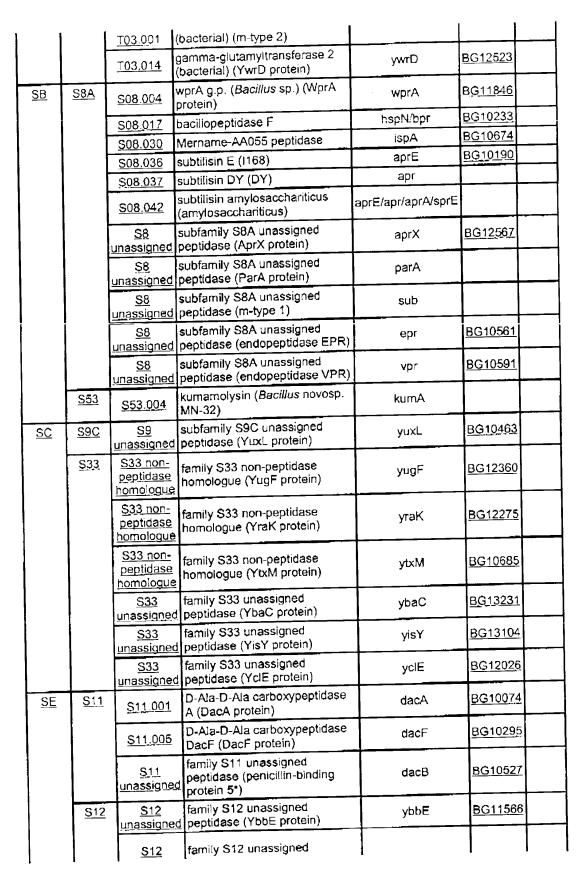
Bacillus subtilis

Page created 30-Aug-2002

	III Gata	base identifi	er: 1423						
Superk	ingdom								
Kingdom		Eubactería							
Phylum Order		Firmicutes							
		Bacillales							
Family		Bacillaceae							
	ASES		120						
Count	of know	n peptidases	and homologues: 120		T				
Clan	Family	1	Peptidase or homologue (subtype)	Gene	Link	Locus			
AC	<u>A8</u>		signal peptidase II	lspA/lsp	BG11793				
<u>AD</u>	A24	<u>A24</u> unassigned	family A24 unassigned peptidase (ComC protein)	comC	BG10323				
CA	<u>C39</u>	C39 unassigned	family C39 unassigned peptidase (sunT protein)	sunT	<u>BG12683</u>				
<u>CF</u>	<u>C15</u>	C15.001	pyroglutarnyl peptidase I (prokaryote)	рср	BG10873				
СJ	<u>C56</u>	C56 unassigned	family C56 unassigned peptidase (GSP18 N-terminal fragment)	yfkM	B <u>G12929</u>				
		C56 unassigned	family C56 unassigned peptidase (YraA protein)	yraA	BG13776				
<u>CX</u>	<u>C40</u>	C40.002	murein endopeptidase lytF (Bacillus subtilis) (YhdD protein)	yhdD/lytF	BG13010	<u> </u>			
		C40.003	lytE g.p. (<i>Bacillus subtilis</i>) (PapQ protein)	LytE/papQ	BG11406				
		C40	family C40 unassigned peptidase (YwtD protein)	ywtD	BG12535	<u>i</u>			
		C40	family C40 unassigned peptidase (YddH protein)	yddH	<u>BG12115</u>	<u> </u>			
		C40	family C40 unassigned peptidase (YkfC protein)	ykfC	BG13233	3			
		C40	family C40 unassigned peptidase (YojL protein)	yojL	BG13564				
MA(E) <u>M3B</u>	_	oligopeptidase F (YjbG protein)	yjbG	BG13130	<u> </u>			
		M3	subfamily M3B unassigned peptidase (YusX protein)	yu s X	<u>BG1403</u>	6			
	<u>M4</u>	M04.012	Inoutral protesse B (Bacillus	пргВ	BG1069				
		M04.014		nprE	BG1044	≝			
	<u>M32</u>		family M32 unassigned	ypwA	BG1145				
	M41			ftsH	<u>BG1013</u>	<u>-2 </u>			







		unassigned	peptidase (penicillin-binding protein pbpX)	рврХ	BG12642
		\$12	family \$12 unassigned peptidase (penicillin-binding protein pbpE)	pbpE	B <u>G10390</u>
	<u>\$13</u>	<u>\$13.001</u>	D-Ala-D-Ala peptidase C (deduced from nucleotide sequence by MEROPS)		
		<u>\$13.002</u>	D-Ala-D-Ala carboxypeptidase (Actinomadura strain R39)	pbp	BG10969
<u>SF</u>	<u>S16</u>	S16.001	lon protease (type 1) (A)	lonA	BG10338
		<u>\$16</u> unassigned	family S16 unassigned peptidase (YlbL protein)	ylbL	BG13364
		S16	family \$16 unassigned peptidase (B)	lonB/lon2	BG11077
	<u>S24</u>		family \$24 unassigned peptidase (\$0\$ regulatory protein dinR)	lexA/dinR	<u>BG10678</u>
	S26A	S26.003	signai peptidase SipS (SipS)	sipS	BG10515
		S26.004	signal peptidase SipT	sipT	BG11977
	į	S26.005	signal peptidase SipU	SipU/YCSB	BG11223
	l	S26.006	signal peptidase SipV	sipV/yhjF	BG12674
		\$26.007	signal peptidase SipP	sipP	
	1	S26.007	signal peptidase SipP (SipP)	sipP/sipP40	
	S26B	S26.011	signal peptidase SipW (Bacillus sp.)	sipW/yqhE	<u>BG11696</u>
SĶ	<u>\$14</u>	\$14,001	endopeptidase Clp (type 1)	clpP/lopP/yvdN	BG19016
<u> </u>		514	family S14 unassigned peptidase (TepA protein)	ymfB/tepA	BG11055
<u>sm</u>	<u>541A</u>	S41	subfamily S41A unassigned peptidase (YvjB protein)	yvjB	BG14110
		S41 unassigned	subfamily S41A unassigned peptidase (CtpA protein)	ctpA/yzbD/orfRM1	
<u>SX</u>	S49	\$49.001	protease IV (Ytel protein)	ytel/sppA	BG13839
<u>071</u>	\$54	\$54	family S54 unassigned peptidase (YqgP protein)	yqgP	BG11683
		S54	family S54 unassigned peptidase (YdcA protein)	ydcA	BG13231
<u>UX</u>	U4	U04.001	sporulation factor SpolIGA	spoliGA	BG10234
<u> </u>	<u>U32</u>	U32 unassigned	family U32 unassigned peptidase (YnN protein)	yrrN	BG13795
		1132	family U32 unassigned peptidase (YrrO protein)	yrrO	BG13796
			ushG protein (Bacillus sp.)	yabG	BG10106

yabG protein (Bacillus sp.)

family U61 unassigned peptidase (YocD protein)

<u>U61</u> family U61 unassigned unassigned peptidase (YkfA protein)

<u>U57</u>

<u>U61</u>

U57.001

<u>U61</u>

unassigned

yabG

yocD

YkfA

BG13517

BG13231

Peptidases of Bacillus subtilis

© 2002 MRC/BB\$RC All rights reserved